253-Pos Board B39

Dynamical Peptide Motions in MHC Groove is Crucial for Recognition and Activation of Certain T Cells

Haruo Kozono[1,2], Naoki Ogawa[2,3], Kentaroh Hoshisashi[2,4], Hiroshi Sekiguchi[2,4], Yuko Kozono[1], Osami Kanagawa[2,4], Yuji C. Sasaki[2,4].
[1]Tokyo University of Science, Noda, Chiba, Japan, [2]JST/CREST SASAKI-team, Tokyo, Japan, [3]Nihon University, Tokyo, Japan, [4]The University of Tokyo, Kashiwa, Chiba, Japan.

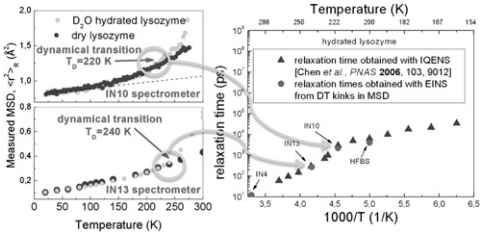
We detected dynamic motions of peptides in MHC II with single-molecule technology using X-rays. T cells see peptides in the context of MHC molecules. This recognition should be specific to each antigens to prevent anomalous self-attack by the activated T cells. However, cross-reactivity of T cell is also well known phenomena. Also, some T cells see peptides/MHC in peculiar forms. As a typical of such peptide/MHC, we studied type B form of I-A^k with HEL peptides, as well as I-A^{g7} with newly found diabetogenic peptides for molecular flexibility. in order to elucidate the mechanism of the recognition, we used diffracted X-ray tracking method (DXT) that monitors real-time movements of individual proteins in solution at the single-molecular level. We found that peptides have its individual Brownian motions that are different from MHC protein themself, and the extent of motion diminishes by time in order of days, which may mimic the DM function. We also found that the rotational motions of peptides correlate with the type B T cell activation or with diabetogenic I-A^{g7} activation. The rotational motion of peptides may create transient conformation of peptide/MHC complex that recognized by a population of T cells.

254-Pos Board B40

Protein Dynamics by Neutron Scattering: The Protein Dynamical Transition

Antonio Benedetto, Federica Migliardo, Salvatore Magazù.
Messina University, Messina, Italy.

Despite recent extensive efforts, the nature of the dynamics of biological macromolecules still remains unclear. in particular, contradicting models have been proposed for explaining the temperature behavior of the mean square displacement, MSD, and of the characteristic system time, τ. To solve this puzzle, different elastic incoherent neutron scattering experiments with different instrumental energy resolutions were performed on dry and hydrated lysozyme [S Magazù, et al. *J. Phys. Chem. B* **2011**, 115:7736]. Characteristic system times have been correlated to the *dynamical transition* (DT) kinks in the MSDs (as is shown in the figure). The obtained results show that the DT is a finite instrumental energy resolution effect, more specifically, it appears when the characteristic system time intersects the resolution time of the spectrometer used; it does not imply any transition in the dynamical properties of the systems, and, it is not due to the fragile-to-strong dynamical crossover (FSC) in the characteristic system time temperature behavior. Furthermore, the obtained results confirm the change in τ(T) at T=220K, and show that it is not due to finite instrumental energy resolution effects and it is not connected to errors in the data analysis protocol.



255-Pos Board B41

Conformational Dynamics of Lipid-Reconstituted LeuT Studied at the Single Vesicle and Single Molecule Level

Marijonas Tutkus[1], Signe Mathiensen[1], Dinna Barthold Kruger[2], Asger Tonnesen[1], Ulrik Gether[2], Claus J. Loland[2], Dimitris Stamou[1].
[1]Bio-Nanotechnology Laboratory, Department of Neuroscience and Pharmacology and Nanoscience Center, Copenhagen University, Copenhagen, Denmark, [2]Molecular Neuropharmacology Group, Department of Neuroscience and Pharmacology, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark.

Conformational changes are an essentially prerequisite for the function of transmembrane(TM) proteins. Ensemble studies typically average dynamics of the conformational changes that consequently become obscure. Therefore an approach for revealing the dynamics of conformational changes is to study each protein at the single molecule (SM) level. At present only two reports have addressed the conformational dynamics of any TM protein at SM level[1,2] reflecting the difficulties and importance of these experiments. Proteins in these experiments were solubilized in detergent micelles. Here we investigate conformational dynamics of the Leucine transporter(LeuT) reconstituted in lipid vesicles, to understand the influence of the membrane on the transporter. We are focusing on single vesicle and SM microscopy measurements of allosteric transitions and oscillations between different states of the sixth TM helix (TM6) of LeuT.

We have developed a unique strategy[3,4,5,6] for immobilization of TM proteins under conditions that minimize non-specific interactions with the surface and thus minimize denaturation. We reconstitute membrane proteins into vesicles, which are anchored on a Neutravidin coated surface with biotinilated lipids. In this manner vesicle thus serves as a 3D scaffold that minimizes protein-surface interactions. By employing this method we have successfully reconstituted LeuT. The protein is labeled with the tetramethylrhodamine (TMR) dye on TM6 (at position 192C), which is quenched by Histidine (position 7H) when LeuT is in an inactive conformation. Variation in distance or orientation between the quencher and TMR, which are induced by the conformational changes of the protein during binding of substrate affect fluorescence signal intensity of the TMR. The dynamics of conformational changes are monitored by Total Internal Reflection microscopy at the single vesicle and the SM level.

256-Pos Board B42

Studying the Differences in Relaxation Activity by Topoisomerase I and III at the Single Molecule Level

Ksenia Terekhova, John F. Marko, Alfonso Mondragón.
Northwestern University, Evanston, IL, USA.

*E. coli t*opoisomerases I and III (Topo I and Topo III) can relax negatively (-) supercoiled DNA, and also catenate or decatenate DNA molecules containing single-stranded DNA regions. Although these enzymes share the same mechanism of activity and have similar structures, they participate in different cellular processes: Topo I is mainly involved in transcription whereas Topo III is involved in recombination, reflected in bulk experiments as a more efficient DNA relaxation activity by Topo I and a more efficient catenation activity by Topo III (1,2). This raises the question: what characteristics of Topo I and Topo III are responsible for their different behavior?

To examine the differences in the activity mechanisms of the two related type IA topoisomerases, single molecule relaxation studies were conducted on several DNA substrates: (-) supercoiled DNA, (+) supercoiled DNA with a 12bp/ 27bp mismatch, and (+) supercoiled DNA with a 12bp/ 27bp bulge. Three major differences between the mechanism of Topo I and Topo III were examined: the time lag before initiation of relaxation events, the rates of DNA relaxation, and the total rates of DNA relaxations. The experiments show differences in the way the two proteins work at the single molecule level, while also recovering the bulk experiments observations. The results provide insights into the mechanism of both proteins, help to understand their differences, and explain why Topo I is more efficient than Topo III in relaxing (-) supercoiled DNA.

1. Tse-Dinh, Y. C., *Bacterial and archeal type I topoisomerases.* Biochim Biophys Acta, 1998. 1400(1–3): p. 19–27.
2. Zhu, Q., P. Pongpech, and R. J. DiGate, *Type I topoisomerase activity is required for proper chromosomal segregation in Escherichia coli.* Proc. Natl. Acad. Sci. USA, 2001. 98(17): p. 9766–71.

257-Pos Board B43

Quantification of Protein Distribution on Liposomes using Confocal Microscopy: A Single Mobile Fluorescent Particle Detection Method

Ouided Friaa[1], Sanjeevan Shivakumar[2], Melissa Furukawa[1], Radhika Voleti[2], Aisha Shamas-Din[2], Brian Leber[2,3], David W. Andrews[2], Cécile Fradin[1,2].
[1]Department of Physics & Astronomy, Mc.Master University, Hamilton, ON, Canada, [2]Department of Biochemistry and Biomedical Sciences, Mc.Master University, Hamilton, ON, Canada, [3]Department of Medicine, McMaster University, Hamilton, ON, Canada.

Bcl-2 family proteins play a crucial role in the regulation of the permeabilization of the outer mitochondrial membrane (OMM), which is one of the main steps leading to cell death through apoptosis.

To a first approximation, apoptosis is controlled by the balance between pro-apoptotic and anti-apoptotic Bcl-2 family proteins. Deregulation of this balance induces a dysfunction of the apoptosis process leading to different pathologies. The molecular mechanism leading to OMM permeabilization is not yet understood in details. This is in part because of the multiplicity and complexity of the molecular interactions involving Bcl-2 family proteins, since each of them can often interact with itself (homo-oligomerization), with other members of the family (hetero-oligomerization) and with the lipids of the OMM.

Whereas in most studies ensemble methods have been used to study these proteins, we have used a single mobile particle detection method to characterize the distribution of different Bcl-2 family proteins on a population of large unilamellar liposomes, in order to better understand the interplay between different family members and lipid membranes during the process of membrane permeabilization. We used large unilamellar liposomes (~ 200 nm in diameter) with a lipid composition mimicking that of mitochondrial membranes and fluorescently labeled recombinant proteins, which were imaged by confocal